IMMUNOLOGICAL DIAGNOSTICS AND DIFFERENTIAL DIAGNOSIS OF LUPUS ERYTHEMATOSUS

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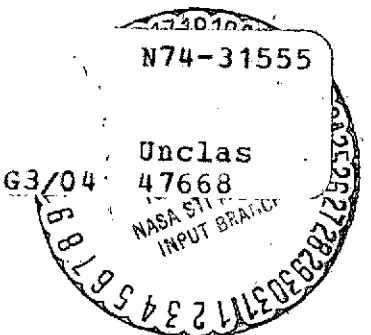

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16. Abstract

Methods in current use for determination and differential diagnosis of systemic Lupus erythematodes are summarized and discussed

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IMMUNOLOGICAL DIAGNOSTICS AND DIFFERENTIAL DIAGNOSIS OF LUPUS ERYTHEMATOSUS

Wolfgang P. Herrmann

If one follows the history of Lupus erythematosus (L. e.) from its beginning to the present, the broadening of the concept of the disease, as it is used today, seems noteworthy: the same term, with which Cazenave (1851) first named a skin affection which was clinically sharply outlined, primarily chronic in course, and benign skin disorder, now serves simultaneously to designate a severe, often life-threatening, general disease of extremely manifold nature, in which the skin lesion which gave the name no longer is an obligate symptom. /209*

The research results of the last 15 years impressively indicate that localized, disseminated and systemic L. e. are, in fact, different courses of one and the same disease (for literature, see Dubois and many others).

This development – it began almost exactly 100 years ago with the first description of acute and subacute courses of L. e. by M. Kaposi (1872) – has raised considerable problems for differential diagnosis. The severe, systemic form of L. e. (SLE) often lacks the major morphological skin symptom. To be sure, skin eruptions can be observed in some stage of the disease in about 80% of all patients with SLE; but these are all too often quite uncharacteristic, both macroscopically

* Numbers in the margin indicate pagination in the original foreign text.

and microscopically [12]. There is also the fact that such disease symptoms, like, in addition, a large part of those which proceed with typical skin lesions, often go for years with the picture of rheumatic polyarthritis before they erupt in feverish outbreaks with visceral or viscero-cutaneous symptoms [12-14]. Thus, laboratory diagnostics, and in particular immunological diagnostics have a very special importance with the systemic forms of L. e., because the success of our therapeutic attempts depends decisively on early diagnosis, before a large degree of irreparable damage has occurred in the internal organs.

The immunological diagnosis of SLE is based on the knowledge that many immunological phenomena occur with this affliction. Today, we already know more than 20 different auto-antibodies which can be demonstrated with variable regularity in the peripheral blood of SLE patients. These include antibodies against red and white blood cells, cell nuclei and nuclear components, various cytoplasmic components, clotting factors, thyreoglobulin, various organ extracts, and many others (Table 1). None of them is strictly disease-specific for L. e., but we do not yet know any other human disease with which they are found in comparable variety.

With respect to diagnosis, the antinuclear factors (ANF) are of particular interest. These are humoral antibodies of type IgG, which are directed against cell nuclei or their components. These antibodies are not organ-specific, and the most important of them are not even species-specific. Of the many methods which have been developed to demonstrate them – Beickert [1] named no less than 30 of them as early as 10 years ago – we shall mention here only some of the most useful.

The indirect immunofluorescence technique of Coons is the most sensitive method of demonstrating them. It is done both in

tissue slices (frozen sections) and in nucleated human and animal blood cells, where the complete cell nucleus serves as the antigen. With the serum of SLE patients, the test is positive in almost 100% of cases.

Another advantage of this method is that the antibody titer in the patient serum can be followed quantitatively by appropriate dilution series. Such curves for the course of the titer are extremely valuable for evaluation of the various phases of the sickness, because the ANF titer rises with increasing severity of the disease and falls again during the remission phase.

Determination of the titer is also important because the demonstration of ANF is not per se disease-specific. Low titers can also be found in healthy persons who are blood relatives of SLE patients, in about 30-50% of patients with chronic discoid L. E., in rheumatoid polyarthritis (about 10-20%), as well as in progressive scleroderma, Sjögren syndrome, many forms of hepatitis, and, in old age, even in samples from clinically healthy patients [13, 15]. To this extent, the immunofluorescence technique for demonstration of ANF, with only qualitative evaluation, is troubled with a relatively high proportion of "false" positive reactions. But titers of 1:64 and more are always suspect for SLE or scleroderma, because such titers hardly ever appear with dermatomyositis and periarteritis nodosa [13]. The usefulness for differentiation of discoid and systemic L. e. is judged differently, depending on the degree to which the diagnosis of SLE is dependent on the demonstration of ANF.

The antiglobulin consumption test of Steffen is no less sensitive. Its sensitivity limit is 0.57 µg antibody nitrogen per milliliter of serum. Lyophilized hog or calf thymus nuclei serve as the antigen. In this method, the consumption of

Table 1. IMMUNE PHENOMENA IN SYSTEMIC LUPUS ERYTHEMATODES

Antigens	Antibodies	Methods of Demonstration
Cell nuclei	IgG	Phagocytosis test
		Antiglobulin consumption test
		KBR
		Fluorescence methods
Nucleoprotein	IgG	KBR
	IgG, IgM	Fluorescence methods
	(DEADE I = VII/VIII)	Hemagglutination
	IgG	Latex agglutination
	IgG	Precipitation
		Passive cutaneous anaphylaxis
DNA	IgG	Precipitation
		KBR
		Hemagglutination
		Bentonite flocculation
	IgM (DEAE VII)	Passive cutaneous anaphylaxis
Histone	IgG	KBR
Nuclear extract (free of DNA and histone)		KBR
Leukocytes	Cohn II + III	Agglutination
Lymphocytes		Intracutaneous test
Cytoplasmic components		Precipitation
Mitochondria	IgG/IgM	KBR
Microsomes		

Table 1, continued.

Antigens	Antibodies	Methods of demonstration
Erythrocytes		
Thrombocytes	Cohn II + III	Agglutination
Rheuma factor	IgM	Waaler-Rose test Latex agglutination
Clotting factors	IgG	
Cardiolipin	IgM	
Thyreoglobulin	IgG/IgM	KBR Latex agglutination
Organs		
Heart	IgG	Passive hemagglutination
Liver	IgG	KBR Passive hemagglutination
Kidneys	IgG	KBR Precipitation Passive hemagglutination
Spleen		KBR
Arteries		Intracutaneous test
Musculature		KBR
Biologically false		KBR
Positive lues reaction		FTA-ABS

KBR: Expansion unknown
FTA: Fluorescent treponemal antibody
ABS: Expansion unknown

incomplete, i. e., non-agglutinating, ANF by the antigen is measured. It is then titrated with an anti-γ-globulin serum. Here, too, there is a relatively high proportion of "false" positive reactions, because the results agree extensively with those of the indirect immunofluorescence test.

The LE cell test is also based on the presence of auto-antibodies against whole cell nuclei. This test tube phenomenon was first described by Hargraves, Richmond and Morton (1948). It is the oldest and at the same time one of the technically simplest methods for demonstration of ANF, because no special laboratory equipment is needed. Various techniques have been reported for preparation of the LE cell preparation. These work partly as a direct and partly as an indirect test with native or heparinized venous blood. It is of decisive importance in all methods that enough nucleated blood cells are disrupted by appropriate manipulations (shaking, stirring, and the like), because the antinuclear antibody is unable to penetrate the intact cell membrane. /210

There are extraordinarily divergent statements in the literature on the frequency with which the LE cell phenomenon is positive in SLE. They vary between 40 and 100%. In our own experience and that of other authors, the test can be positive with about 75-85% of SLE sufferers. In any case, it is distinctly less sensitive than the two methods mentioned previously, because LE cells form only when the concentration of the liberated antibody exceeds 10 μg antibody nitrogen per milliliter of serum [16]. This, to be sure, has the advantage that one has to accept "false" positive results less often. A disadvantage is that the test allows no quantitative statements, even though the density of the LE cells in a smear very often is correlated with the severity of the disease. The number of LE cells rises during an acute outbreak, while it decreases during the remission phases.

Aside from the methods which have been named, there is a series of other serological reactions available for demonstration of antinuclear factors. With some of them, quite different autoantibodies can be demonstrated. The results obtained cannot be compared directly with each other. Most of them are only of subordinate importance for routine diagnosis of SLE. Only the latex-nucleoprotein test and the DNA-bentonite flocculation reaction have become widespread.

The latex-nucleoprotein test is an agglutination reaction in which a suspension of nucleoprotein-coated polystyrene latex particles is used as the antigen. Such suspensions are commercially available. The test can be done within a few minutes on a microscope slide and read macroscopically. Thus, it can also be used without anything else in general practice. Admittedly, its sensitivity is not very high, and false positive reactions are obtained relatively often, particularly with syphilis [1, 8]. Thus, the latex particle test is not as suitable as a routine method for demonstration of antinuclear factors as for a check on the course of the disease, when the presence of SLE has been confirmed with more reliable methods.

The bentonite flocculation reaction was developed specifically for demonstration of antibodies against deoxyribonucleic acid (DNA), which are particularly characteristic of SLE. Here, the antigen is a suspension of DNA-coated bentonite particles (bentonite is a strongly adsorbing clay mineral), which flocculate on addition of serum containing antibodies to DNA. The reaction is considered very specific, but it has only limited diagnostic value because antibodies to DNA occur only in part of the SLE patients. Bertrams et al. [2] have recently developed a DNA-bentonite-Coombs tests which is supposed to be even more sensitive.

Investigation of the fine tissue of the skin offers another possibility for immunological diagnosis. In L. e., immune complexes of IgG, IgM and complement are deposited at the epidermis-corium boundary in the region of the efflorescences, and in SLE occasionally also in the clinically healthy skin. These can be detected with the direct immunofluorescence technique of Coons and Kaplan. Burnham and Fine (1969) have described three fluorescence patterns. In the chronic skin lesions with atrophy and hyperkeratosis, one finds a wide fluorescence band below the basal membrane with great regulatity. It is extremely characteristic. In fresh, more erythematous and edematous efflorescences, on the other hand, and in the clinically unchanged skin of SLE, the fluorescence is more thread-like or spotted. Similar, but still morphologically distinguishable, fluorescence patterns are found otherwise only in bullous pemphigoid. This phenomenon, first described by Burnham et al. (1963), and repeatedly confirmed since them, is practically always demonstrable with discoid L. e., but not so regularly with SLE.

Most authors, therefore, are of the opinion that this histological immunofluorescence finding is so characteristic that it can be used for differential diagnosis versus polymorphic eruptions from light, Jessner-Kanof lymphocytic infiltration, dermatomyositis, progressive scleroderma, and many others.

To be sure, Jablonska et al. have reported certain doubts in this respect, because in their experience such deposits can be found wherever telangiectases are present – from the same cause, and also in rosacea, dermatomyositis and progressive scleroderma. Winkelmann et al. (1972) have also been able to demonstrate immune complexes at the epidermis-corium boundary in dermatomyositis, although these gave only comparatively weak fluorescence and contained

/ 211

primarily IgA and IgM, and rarely IgG. The differential diagnostic value of the direct immunofluorescence-histology can, therefore, not be evaluated quite as highly for SLE as for discoid L. e.

A large series of good and repeatedly confirmed laboratory methods are available to us. They can contribute to clarification of the diagnosis in doubtful cases. But one should remember that none of them are absolutely disease-specific, so that in the individual case one must always test whether the results of the laboratory diagnosis agree with the clinical picture and course.

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